REMARKS

Reconsideration is requested.

Claims 1-106 have been canceled, without prejudice. Claims 107-135 have been added and are pending.

Appeal No. 2005-0397 has been abandoned, without prejudice, upon filing of the attached RCE.

The Examiner will appreciate that claims 107-119 are largely based upon now-canceled claim 106 wherein the nature of the cell has been specified as *E. coli*. The claims are submitted to be supported by the application as filed. The Examiner is believed to have acknowledged in point [5] on page 4 of the Office Action of May 2, 2003, that certain embodiments were enabled by the specification. Specifically the 354 mutation of *Photinus pyralis* and the equivalent Luciola mutation, are believed to have been recognized as having been supported on the basis of at least the reference in the specification to WO95/25798. The specification also includes a reference to European Patent Application No. 92110808.0, which is published as EP-A-524448, and which is a patent application corresponding to the cited Kajiyama reference, such that the applicants believe these proteins are also supported by the disclosure. A copy of the front page of this reference is attached.

The nature of the luciferase and the adenylate kinase are defined in claim 107 in a manner the applicants believe will be recognized by one of ordinary skill as having been described and enabled by the specification. Proteins described in a manner recited in claim 107 are believed to be known in the art, as listed in the specification and art which has been produced subsequently. In this respect, the Examiner's attentions is

drawn also to subsequent publications such as WO 99/14336, WO 01/2002, WO 00/24878 and WO 01/31028, all of which relate to thermostable luciferase mutants. It is clear that the method of the present invention could easily be applied to production of these proteins. Restriction of the scope of the claims to these specific examples only would mean that the applicants' invention could be readily utilised by others without recourse to the applicants, which the applicants believe would be inequitable. Copies of the front pages of these references are attached for the Examiner's convenience.

Claims 120-127 represent a further group of claims, which the Examiner is believed to have acknowledged to be enabled by the description.

Claims 128-135 are also submitted to be supported by the specification and the reference to the Kajiyama European application which is referred to on page 8 line 22 of the specification.

The claims are submitted to be patentable over the art of record.

Specifically, EP-373962 is not concerned with luciferase production. It describes the expression of highly thermostable proteins (essentially those from thermophilic organisms which do not express luciferase as far as the applicants are aware) in otherwise unmodified mesophilic organisms. The extreme thermostability of these products means they can be purified by heating to levels at which all the other host cell proteins are denatured.

This teaching lacks, for example, any suggestion that the host cells should be further modified to increase the thermolability of any particular contaminant protein. The reference is believed to not provide such a suggestion because the levels of thermostability encountered in the proteins produced here is so high, that the product

may be safely heated to temperatures, such as 70°C, at which all normal *E.coli* cell proteins are denatured. Therefore any such considerations would be entirely unnecessary, and so there is nothing to motivate a skilled person to do this. None of the additional art of record cures this deficiency.

Kajiyama, is understood to teach that a particular mutated form of luciferase has a greater thermostability than the wild-type protein, where the extreme sensitivity to temperature presents real problems in terms of storage etc. of the product. As the Examiner has previously noted, Kajiyama reports that the mutant enzyme maintained 30% of its activity when heated at 50°C for 40 minutes. The applicants believe that this is not the sort of extreme thermostability that would allow the method of EP-373962 to be applied to its production. Even under these temperatures, 70% of the product appears to have been lost. This would not have motivated an ordinarily skilled person to consider applying the method of EP-373962 to the Kajiyama protein, where even higher temperatures for longer periods would have been involved. The applicants believe that there would have been no reasonable expectation of successfully obtaining any usable product.

The remaining previously-cited art is believed to have been combined with hindsight as, neither Gilles nor Belinga are concerned with the expression of recombinant proteins.

Specifically, Gilles, is a scientific paper, which is understood to be concerned with the structure-activity relationship of *E. coli* adenylate kinase. In the course of the study, they found incidentally, that certain mutations led to mutants where the adenylate

kinase was more thermolabile than in the wild-type. They are not believed to have suggested any particular applications for this mutant as an expression host.

Belinga is understood to describe how it is preferable to remove firefly adenylate kinase from preparations of luciferase obtained from fireflies. At best therefore it teaches that this is an undesirable type of contaminant in a luficerase product. However, it suggests that it can be removed using conventional protein purification methods. There is nothing in this reference to motivate an ordinarily skilled person to look for ways of removing specifically this contaminant from a recombinant product by using specifically engineered cells.

In effect, none of the references are believed to suggest luciferase purification by specifically engineering an exploitable "space" in the relative thermostabilities of the luciferase and the adenylate kinase (or indeed between any protein and a specific contaminant). The claims are submitted to be patentable over the art of record.

The Examiner is requested to contact the undersigned if anything further is required.

Respectfully submitted,

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